Statement of
Tobacco Institute Testing Laboratory Methodolgy

The Tobacco Institute Testing Laboratory obtains samples of the cigarette varieties to be tested from the same (50) 1 locations, at the same time, and in the same quantity (2 packs delete) as the Federal Trade Commission. Western Union procures samples simultaneously with its procurement of samples for the Commission.

When samples are received at the Tobacco Institute Testing Laboratory their place of origin is recorded. The cigarettes are then inventoried and stored in unopened packages in the sample conditioning room according to variety.

All subsequent handling of the cigarettes preparatory to actual smoking is performed in areas maintained at 75°F +? and 60% +? relative humidity.

In preparation for testing of a market survey, the packages are opened and cigarettes chosen at random. The number of cigarettes selected is based upon the number of packages received by the Tobacco Institute Testing Laboratory. It is sufficient in all cases to provide between 160 and 240 cigarettes for final selection. At the same time, an identical sample is also drawn and reserved for emergency use.

The first sample of between 160 and 240 cigarettes of each variety is placed in the sample conditioning room (delete) on →

screens labeled by variety. The cigarettes selected as the reserve sample are sealed in polyethylene bags and kept in cold storage where they are available if needed during the testing. The remaining cigarettes, still in packages, are also sealed in polyethylene bags and placed in cold storage.

Prior to sample selection, the cigarettes are marked to the appropriate butt lengths. ~~When the desired butt length is other than 30 mm. it is determined from overwrap measurements on randomly selected cigarettes.~~ 23mm or tipping + 3 whichever is the longer If obvious differences in the length of the tipping paper (filter overwrap) occur among cigarettes in the same sample the cigarettes are separated, marked accordingly, and remixed. A ~~machine (device)~~ manufactured by Phipps & Bird, Inc., Richmond Virginia, is used to mark the cigarettes conveniently, rapidly and accurately.

Individual ports of five cigarettes each are then selected at random, inspected for any damage, and placed in a beaker marked to identify order of smoking. Prior to actual smoking, each load is verified to insure no errors were made in selection or loading. Cigarettes which show such obvious flaws as loose ends or torn cigarette paper are replaced. The order of smoking for each of the 20 ports of each variety is determined by a system of random selection designed to insure that smoking of each variety will be interspersed throughout the entire testing period. This guarantees

that testing results for any one variety will not be biased by temporary variations in testing conditions.

The basic procedure for collecting total particulate matter is the Cambridge filter pad technique developed by Wartman and coworkers.*/ Immediately prior to use the assembled filter holders are tested for leaks to insure that air will be drawn only through the cigarette. Each filter holder is also weighed on an analytical balance to the nearest 0.2 mg.

The cigarettes are then smoked on one of two ~~identical~~ smoking machines built for the laboratory by Philip Morris Incorporated. The machines are of the type developed by Philip Morris and are mechanically ~~identical~~ similar to those now commercially manufactured by Phipps & Bird Inc.

Smoking is done in a specially ~~prepared~~ conditioned room where temperature and humidity are carefully controlled at 75 ± 2° Fahrenheit and 60 ± 2% relative humidity.

Flow of the air out of the machine is controlled by top vents and exhaust fans. Settings controlling the air flow are checked periodically. The automatic controls for temperature and humidity are regularly verified against standard temperature and humidity measuring devices.

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*/ Analytical Chemistry, Vol. 31, pp. 1705-1709 (1959).

The smoking machines are set at the ~~Ogg~~ parameters*/ of 60 ± 1 second puff frequency and 2 ± .2 seconds puff duration, as noted in TOAC. delete The ~~Ogg~~ parameter for puff volume, 35.0 ± .5 cc, is refined to operate at the more precise setting of 35.0 ± .2 cc. Volume settings are checked each day. The puff frequency and duration are checked regularly to assure uniform machine performance. Tests are also made to insure that there are no leaks in the smoke train.

Cigarettes are sealed into the filter holder by means of rubber sleeves with an inside diameter of 5/16". In the case of thin cigarettes a rubber sleeve with an inside diameter of 1/4" is employed. These sleeves are prepared by cutting approximately 1/2" sections from surgical tubing.

Twenty delete (20) cigarettes are smoked by the machine at ~~once.~~ one time delete Each smoke delete run includes 4 monitor cigarettes smoked at positions on the machine that are selected at random. All 20 cigarettes in a given smoking are simultaneously ignited by electric lighters. The Philip Morris machine allows smoking to be automatically stopped at the desired butt length by placing a string across the cigarette at the ~~point~~ butt length delete previously marked. A switch is triggered when the string is burnt that cuts off further smoking at that point.

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*/ Journal of the A.O.A.C., Vol. 47, No. 2 (1964).

After smoking is completed the holder and pad are reweighed on the analytical balance. Total particulate matter is determined by calculating the difference in weight between the Cambridge filter pad and holder before and after a sample of 5 cigarettes has been smoked on it.

After reweighing, the holder is disassembled and the pad removed. In removal the pad is first carefully folded over with forceps and then used to wipe clean the inside of the holder. This insures that any remaining particulate matter is deposited on the pad. The pad is placed in an Erlenmeyer flask that previously has been washed thoroughly and allowed to dry for at least two hours in an oven set between 130° and 150° C. After removal from the oven the flask is sealed while still hot with a serum type rubber stopper. An identifying label is afixed prior to use. After placing the pad in this flask, the flask remains sealed except when opened to add solvent for nicotine and water extraction.

The filter holder is cleaned by wiping with a cotton pad dampened with 2-propanol and dried with disposable tissues. It is then reassembled with a new filter pad and conditioned in the sample conditioning room.

To analyze the moisture content of the TPM, the Tobacco Institute Testing Laboratory employs the basic method

described by Sloan and Sublett */ and modified by Schultz and Spears. **/ However, further improvements in extracting solvent, equipment, and calibration have been adopted are employed.

The Tobacco Institute Testing Laboratory uses 2-propanol as the extracting solvent and adds 4 ml. of ethanol per 1,000 ml. of solvent. This serves as the internal standard for the gas chromatographic ~~processing~~ analysis. This solvent combination has the advantage that peroxides do not form on standing. It also does not cause the filter pad to disintegrate during shaking. This is an advantage in the subsequent determination of nicotine.

After the solvent is added, the flask is thoroughly shaken for at least an hour to insure that all the water and nicotine get into the solution. A wrist action shaker manufactured by Burrell is employed for this purpose.

The water content of the solution is determined by a gas chromatograph, equipped with an electronic digital integrator. This equipment automatically accumulates and prints the results of each test, thereby eliminating the human measurements required when reliance is placed on the

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*/ Tobacco Science IX, pp. 70-74; Tobacco Vol. 160, No. 17, pp. 28-32 (1965).

**/ Tobacco Vol. 162, No. 24, p. 32 (1966).

mechanically drawn recorder curves. In addition, the Tobacco Institute Testing Laboratory utilizes an electronic calculator programmed to process the results obtained from the integrator. In this manner final figures are made immediately available for tabulating. The procedure insures that the results will be ascertained with accuracy and speed.

To further insure complete accuracy, standards for calibration are derived on an absolute basis by weighing pure water in a clean, dry volumetric flask and diluting the water with solvent. For purposes of calibration each standard is analyzed at least three times, and the moisture value is obtained using unbiased, statistical methods rather than the cumbersome, inaccurate, graphic process.

The analysis for total alkaloids is essentially as outlined in the paper by Ogg*/ with modifications to reduce exposure of the operating personnel to organic vapors and to solvent contact. To eliminate these hazards, each Griffith still unit is equipped with a small funnel in which the sample is prepared. The sample is added by the turning of a valve and rinsing with a small amount of 2-propanol.

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*/ Journal of the A.O.A.C., Vol. 47, No. 2 (1964).

A reduced sample size of 4 ml. is used. This is conveniently drawn from the Erlenmeyer flask and measured using pipette and pipette filler manufactured by Keyes Scientific Company, Cambridge, Massachusetts.

The nicotine, using the reduced sample size, can then be collected in a 250 ml. volumetric flask at an ideal concentration for final measurement. The flask is placed on a magnetic stirrer and mixed to insure a uniform solution of nicotine and water.

The final step in the nicotine analysis is the calculation of the nicotine based on the amount of ultraviolet light absorbed. The Tobacco Institute Testing Laboratory employs for this purpose a spectrophotometer manufactured commercially by Beckman Instrument Company as Model DBG. Standard accessory items are used to permit rapid filling and emptying of the sample container without removal from the instrument. Another item displays the instrumental measurements in easily read form.

As the data are obtained they are entered into a programmed calculator. The final results are automatically computed and then made available for tabulation and verification.